Gastric pH in Rats: Key Determinant for Preclinical Evaluation of pH-dependent Oral Drug Absorption

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Abstract: Data on gastric pH in rats to be used in preclinical models for pH-dependent drug absorption are still limited or contradictory. The aim of this study was to describe gastric pH in rats at fasted state and to evaluate its changes induced by pentagastrin or omeprazole in order to mimic gastric pH at fasted and fed human subjects. Twenty Wistar rats, fasting for 12 h, were randomly assigned into four treatment groups (n=5): control, pre-treated with omeprazole 2 h before pH measurement, pre-treated with omeprazole 12 h before pH measurement, and pre-treated with pentagastrin 20 min before pH measurement. An incision on the stomach wall was made in anesthetized animals, and pH of gastric juice was measured. The observed pH values were significantly different among groups (p=0.0341), with the median (IQR) values of gastric pH of 3.5 (2.7–4.2), 6.7 (4.7–7.0), 5.6 (3.5–6.4) and 2.2 (1.6–3.1) in control, omeprazole 2 h, omeprazole 12 h and pentagastin group, respectively. We recommend using short interval pentagastrin and 2 h omeprazole pre-treatment in fasting animals to model similar gastric pH as is expected in human fasted and fed state pharmacokinetic studies, respectively.

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Introduction

Although animal models are extensively used in the preclinical testing of drugs, sparse work has been reported on evaluating rats as a preclinical model for pH-dependent absorption studies (McConnell et al., 2008; Lubach et al., 2013). Gastric pH in fasted state is generally reported higher (3.9) in rats as compared with men (1.7) (Dressman et al., 1990; McConnell et al., 2008). Previous animal studies also provided conflicting results with respect of impact of food and other factors (Eastman and Miller, 1935; Ward and Coates, 1987; McConnell et al., 2008; Lubach et al., 2013). Moreover, previously published studies report wide inter-individual and intra-individual variability of gastric pH in rats (Fleisher et al., 1999).

Many drugs possess pH-dependent dissolution, solubility, or penetration through biological membranes (Lubach et al., 2013; Yasumuro et al., 2018) that is likely to affect the drug absorption after oral administration. Therefore, preclinical assessment of drug pharmacokinetics may be challenging.

The pentapeptide pentagastrin is well-known to stimulate gastric acid secretion in mammals and is widely used to increase acidity and reduce inter-individual variability in animal pharmacokinetic studies (Lubach et al., 2013), while omeprazole is a proton-pump inhibitor widely used for its potent inhibitory effect on gastric acid secretion (Larsson et al., 1983).

The aim of this study was to describe gastric pH in rats at fasted state and to evaluate its changes by pentagastrin or omeprazole to more closely mimic gastric pH at fasted and fed human subjects.

Material and Methods

Chemicals

Pentagastrin was purchased from Sigma-Aldrich (Prague, Czech Republic) and omeprazole was used as Helicid 40 Inf (Zentiva, Prague, Czech Republic). Ketamine and xylazine were used as Narkamon 100 mg/ml inj sol and Rometar 20 mg/ml inj sol (Bioveta, Ivanovice na Hané, Czech Republic), respectively.

Animals

Male Wistar rats (Velaz, Prague, Czech Republic) were used throughout the study. They were maintained under standard conditions (12-h light-dark cycle, 22 ± 2 °C temperature and 50 ± 10% relative humidity) and fed on water and standard granulated diet *ad libitum*. Twelve hours before gastric pH measurement animals were fasted, with free access to water, and were housed on a grid. All experiments were performed in accordance with the Guiding Principles in the Use of Animals in Charles University, First Faculty of Medicine, and every effort was made to minimize animal suffering. The experimental animal project was approved by the Ministry of Education, Youth and Sports of the Czech Republic under the negotiation number MSMT-9445/2018-8.

Table 1 – Pretreatment schedule in the study groups

Group	Treatment
Control	no treatment
Omeprazole 2 h	omeprazole 20 mg/kg i.p. 2 h before pH measurement
Omeprazole 12 h	omeprazole 20 mg/kg i.p. 12 h before pH measurement
Pentagastrin	pentagastrin 0.25 mg/kg s.c. 20 min before pH measurement

Experimental design

The rats were randomly assigned into four treatment groups (Table 1). The control group were fasted overnight without treatment. The other groups were treated with intraperitoneal omeprazole 20 mg/kg two hours before pH measurement, twelve hours before pH measurement, and subcutaneous pentagastin 0.25 mg/kg twenty minutes before pH measurement, respectively.

Dissection procedure and gastric pH measurement

The anesthetized rat (ketamine 100 mg/kg i.m. and xylazine 5 mg/kg i.m.) was placed on its back with its tail toward the investigator. The abdominal cavity was opened with a V-cut made through the abdominal wall starting at the base of the abdomen and proceeding diagonally across each side to the dorsolateral edge of the thorax. The skin flap was moved onto the chest and the stomach was located. The antrum cardiacum and pylorus of the stomach were ligated. An incision to the stomach wall was made, and gastric juice pH was measured with a pH meter S2K712 (Isfetcom Co., Ltd., Shimokayama, Japan). The pH meter was previously calibrated at two points using standards solutions of pH 4.0 and pH 7.0.

Statistical analysis

Median and interquartile range (IQR) values were calculated using MS Excel 2010 (Microsoft Corporation, Redmond, USA). Significance of differences in gastric pH between the groups was determined by the Kruskal-Wallis test using GraphPad Prism 3.02 (GraphPad Software, Inc., La Jolla, USA). Statistical significance was accepted at $p<0.05$.

Results

Twenty rats weighting 275–379 g were enrolled in this study; five rats per group. Measured gastric pH values in each group are presented in Figure 1. The observed pH values were significantly different among groups ($p=0.0341$), with the median (IQR) values of gastric pH of 3.5 (2.7–4.2), 6.7 (4.7–7.0), 5.6 (3.5–6.4) and 2.2 (1.6–3.1) in control, omeprazole 2 h, omeprazole 12 h and pentagastin group, respectively.

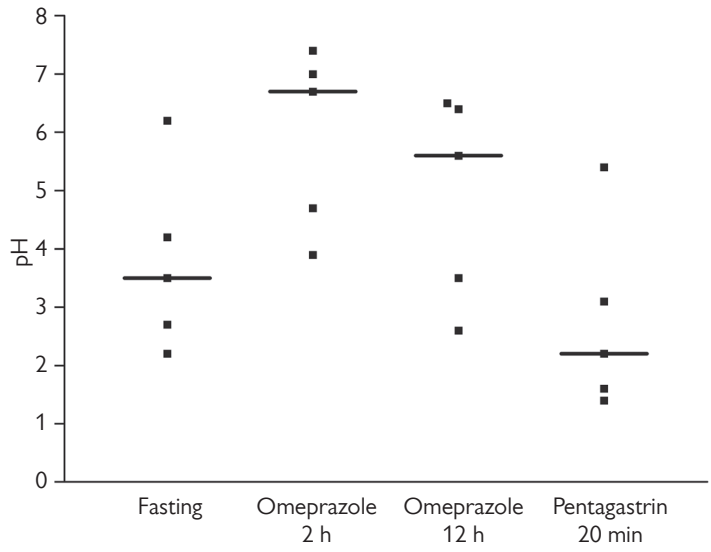

Figure 1 – Individual gastric pH values in fasted rats and rats treated with omeprazole and pentagastrin.

Discussion

We conducted this study to propose experimental conditions for subsequent preclinical pharmacokinetic studies with respect to gastric pH similarity to man.

Despite high inter-individual variability, median as well as minimum and maximum pH values increased in the following order: pentagastrin group, control group, omeprazole 12 h group, omeprazole 2 h group.

The pentagastrin group had similar median gastric pH to that reported in humans at fasting state (Dressman et al., 1990). Although the pH decreasing effect of pentagastrin was expected based on its known mechanism of action, previously published study by Lubach et al. (2013) did not show relevant impact of pentagastrin pretreatment on gastric pH that might be caused by unusually low control fasted state pH. Since pentagastrin is a compound with fast onset of action and short elimination half-life, the longer time interval between pentagastrin administration and pH measurement as compared with our study could also contribute to previously reported lack of pentagastrin effect.

Omeprazole pretreatment 2 hours before pH measurement increased the gastric pH to the range that is comparable to pH seen in man in fed state (Dressman et al., 1990) and similar omeprazole effect on pH in rats has been published recently by Yasumuro et al. (2018). However, although omeprazole is an irreversible proton pump inhibitor that maintains more than 50% of the effect at 24 h after dosing, the 12 h interval already lead to observable diminished omeprazole activity.

Numerous preclinical studies reported administration of drugs at fasting conditions based only on the fact that animals were food-deprived for specified time. It should be noted that we observed undigested wood shavings, fur, or excrements in the stomach of approximately half of the animals even when housed on a grid for the

whole 12 h fasting period before pH measurement. Thus, the animal model may mirror gastric pH with the standard human conditions at pharmacokinetic studies, however, the fasted state in cooperating human subjects might not be fully modelled in large proportion of animals with respect to potential effect of gastric content on gastric motility, its buffering and adsorption effects etc.

Conclusion

Based on our results, we recommend using short interval pentagastrin and 2 h omeprazole pretreatment in fasting animals to model similar gastric pH as is expected in human fasted and fed state pharmacokinetic studies, respectively.

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